Model for MCMV Disease

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Murine cytomegalovirus (MCMV) is a natural pathogen of mice that is present in all wild mice populations and has been used extensively as an animal model for human cytomegalovirus disease. By manipulating the mouse strain, the type of virus used, the inoculation route and the addition of various chemotherapeutic treatments (e.g. immunosuppression), many human diseases can be modeled in a realistic and consistent way. This entry describes the many ways that MCMV has been used to model human disease.

Keywords: congenital disease ; placenta ; salivary gland

1. Introduction

It has famously been stated that “mice lie and monkeys exaggerate”[1], but the use of animal models in the study of infectious disease provides strong evidence for the mechanisms underlying the pathogenesis of infections in humans and provides opportunities to study interactions between hosts and pathogens that are not provided by collecting minimally invasive human samples such as blood, feces, saliva and urine. When investigating infectious diseases, the ability to correlate tissue histopathology with pathogen replication rates over time or evaluate cell signaling and related immune modulation in the context of a complete immune system is of inestimable value and provides mechanistic information, as well as pre-clinical opportunities to test treatment efficacy and toxicity. The ability of a pathogen to infect both mice and humans allowed the development of Koch’s postulates[2], an evidence framework still used today to prove the association of newly described pathogens with infection and subsequent pathological sequelae, albeit with modifications to account for the presence of pathogens in commensal flora[3] and viruses that are attenuated in a culture[4]. An example of this modern reasoning has been applied for SARS-CoV-1[5].

There are many animal models where laboratory-inoculated animals exhibit symptoms or pathogen replication that mimics human infection, even though infection may not occur in the natural environment. These models are useful in carefully evaluated (and disclosed) circumstances (discussed in[6] for SARS-CoV-2) and have provided useful information on infection pathways and the effects of opportunistic infections and potential drug targets[7]. Examples of animal models in non-target species are those developed for influenza and respiratory syncytial virus in ferrets[8] and Ebola virus infection, which have been modeled in suckling (but not adult) immunocompetent mice as well as Syrian golden hamsters[9]. These models and others like them have illuminated pathways used by pathogens to travel from the site of infection to organs or other sites of tropism, as well as immune responses, symptom and infection profiles and treatment options.

In animal models for viral disease, the presence of the correct receptor used by the virus to enter a cell is critical and needs to be largely conserved between species for cross-species infection to occur[6]. For infections where viruses do not naturally infect another species due to absence of the correct receptor, transgenic mice have been developed that allow an animal model to be developed (e.g., for poliovirus)[10]. Some viruses, such as influenza, naturally infect multiple species (avian species, pigs and humans[11]) while others, such as cytomegalovirus, are species-specific. Animal models where the pathogen naturally infects the animal host and the disease is naturally present in wild populations can provide insights into human disease by illuminating complex host-pathogen interactions and provide clinically relevant data.

Cytomegaloviruses (CMVs) from the family Herpesviridae and the subfamily Betaherpesvirinae are species-specific viruses and have been isolated from many mammalian species (e.g., human, mouse, rat, guinea pig and various primates; see[12] for a discussion). CMVs have evolved with their hosts[13] and generally do not replicate completely in vivo in different species after inoculation, even where in vitro growth has been described (e.g., murine cytomegalovirus (MCMV) will grow in vitro in rat cells but will not grow in vivo in rats[14]). However, there have been reports of the replication of disparate CMV in other species in circumstances where xenotransplantation has taken place and the recipient has received immunosuppressive chemotherapy[15][16]. Juvenile rats have also been reported to support the growth of MCMV, although older rats (>6 weeks old) were not susceptible to infection[17].

2. MCMV as a Model for HCMV Infection

The recorded movement of MCMV between organs differs, depending on the route of infection (described in[33] and [12]). There is a strong tropism for the salivary gland, and active viral replication persists in salivary glands for longer than other organs, regardless of the route of infection used. As with all herpesviruses, MCMV infection has a latent phase[32]. MCMV infection without significant manipulation of the host has successfully been used to model various aspects of human infection (Table 1). Information from this natural animal model has greatly improved the understanding of the pathogenesis of HCMV infection in humans.

Table 1. Human disease caused by human cytomegalovirus (HCMV) modeled by murine cytomegalovirus (MCMV) infection in mice.

Human Condition	Lab Conditions	SGV [1]/TCV [2] PFU [3]	Effect	Reference
Viremia	Intraperitoneal (i.p) inoculation of BALB/cByJ mice.	SGV/TCV not specified 10^6 PFU	White blood cells have viral DNA but no evidence of *ie1* RNA.	[34]
Viral latency	BALB/c footpad inoculated at 2 weeks of age. Latency present after 3 weeks.	TCV 10^5 PFU	Whole body irradiation leads to reactivation of infection. Antibody protects from viral dissemination.	[35][36]
Pneumonitis	1. Intranasal inoculation into outbred Swiss mice or intra-tracheal infection of BALB/c mice.	TCV $>10^4$ PFU	Severe diffuse interstitial pneumonitis closely resembling that seen in immunocompromised patients and in newborn infants, 20% died.	[37]
	2. Inoculation of newborn BALB/c.	SGV, 6 PFU i.p.	Pneumonitis and myocarditis, 95% lethal.	[38]
Hepatitis	i.p inoculation of BALB/c mice.	SGV, 10^5 PFU	Hepatitis evident, dose is Lethal $Dose_{50}$.	[26]
Ocular infection (retinitis)	1. Intraocular inoculation (scarified cornea or through corneal limbus) of IRC/Sic mice.	SGV, Tissue Culture Infectious $Dose_{50}$ values given.	Different effects SGV vs. TCV.	[39]
	2. Intraperitoneal inoculation of BALB/c mice.		Inflammatory response in retina (virus not present) and iris (virus present).	[40]
Excretion of CMV into breastmilk	Acute or latently inoculated C57BL/6 mothers (i.p.), leukocytes from BM positive by *ie1* mRNA detection, RTPCR.	SGV 3×10^2–3×10^4 PFU	Evidence of neonatal infection via breast milk. Inoculation of milk into CD-1 1-day old mice results in infection.	[41]
Arterial blood pressure post CMV infection	i.p. inoculation of 2-week-old C57BL/6 mice.	TCV, 3×10^5 PFU/1mL/mouse	MCMV increased blood pressure independent of diet. Increased serum IL-6, TNF-alpha, and MCP-1.	[42]
Viral myocarditis	i.p. inoculation of C57BL/6 and BALB/c mice.	SGV 10^4 PFU	Inflammatory foci in the heart and infection of cardiac myocytes.	[43]
Infection post bone marrow transplantation	Irradiated BALB/c mice inoculated with virus prior to intravenous (i.v.) purified bone marrow cells.	10^5 PFU	Failure in haematopoiesis, leading to death.	[44]
Sexual transmission of CMV via semen	Spermatozoa plus Smith MCMV artificially inseminated (compared with sperm alone).[47]	SGV 10^5 PFU	Embryos collected on E14. One produced cytopathic effect (second passage). No significant difference with numbers or abnormalities.	[45]

[1] Salivary gland derived virus. [2] Tissue culture-derived virus. [3] Plaque-forming units.

3. Mouse Strain Selection Affects the Severity of MCMV Pathogenesis

Investigations of the effect of MCMV in different mouse strains have been pivotal in the modeling of different disease states. Initial work focused on LD_{50} calculations (e.g.,[46]), and it was found that the H-2 alleles of different mouse strains determined their response to infection, including the production of autoantibodies[47] and the induction of myocarditis[48]. Other mechanisms for resistance to MCMV, such as that demonstrated in C57BL/6 mice compared with BALB/c mice or the differing resistance of New Zealand Black and White mice to MCMV infection, were associated with differences in innate natural killer (NK) cell activation and were strongly associated with particular strains of viruses[49][50]. Where MCMV was used as a vaccine vector expressing the mouse ovarian glycoprotein zona pellucida 3 in studies investigating immune-mediated contraception, the specific m157 (viral ligand) to Ly49H (NK cell activation receptor) rapid response to infection was broadly associated with vaccine success[51]. This effect was abrogated through the use of a different virus strain, G4 (isolated from the salivary glands of a mouse from Geraldton, WA[52]), as the vaccine vector. G4 does not have the same interaction with NK cell activation receptors, and this demonstrates the importance of vector strain selection in the development of recombinant CMV-based vaccines[53].

4. MCMV-Based Models for Human Disease Requiring Chemical, Genetic or Physical Manipulation

A recent systematic review and metanalysis calculated that the worldwide seroprevalence of HCMV is 83%[54]. Increasing CMV disease is broadly associated with improvements in medicine because it is often associated with acute immunosuppression, allowing the reactivation of a latent infection. One of the more serious sequelae of CMV infection is found in solid organ transplant recipients, who often experience reactivation from latency and associated pneumonitis, hepatitis and potential organ rejection (discussed in[55]). The likelihood of severe CMV disease increases when the transplanted organ is from a seropositive donor (previously infected with CMV, with no active viral replication but a strong CMV-specific humoral response) being transplanted into a seronegative recipient[56]. In general, outcomes can be improved with the use of antiviral therapy, with a recent metanalysis suggesting that prophylactic treatment using low doses of valganciclovir provides improved outcomes in kidney transplant recipients [57]. CMV can also cause post-transplantation disease in recipients of allogeneic hematopoietic stem cell transplants, and pre-emptive therapy is often initiated after clinical evidence of CMV reactivation (prior to fulminant disease). The economic burden of this therapy is marked[58]. The mouse model of MCMV has been integral in the prediction of useful therapeutics for these clinical circumstances (reviewed in[59], with a discussion of the appropriate use for this model in reliably predicting human outcomes).

In order to model CMV-associated diseases occurring due to immunosuppression, such as retinitis or post-transplantation reactivation, the animal model needs to be manipulated to ensure similarity to human infection. The modification can be due to chemical administration (e.g., corticosteroid use), genetic modification of either the virus or the mouse strain used (e.g., MCMV-deleted m157 virus (Δ157) allows C57BL/6 mice to be used without NK cell activation by the virus) or physiological treatment such as surgery. These modified models are listed in Table 2.

Table 2. Models for CMV disease, requiring significant laboratory manipulation.

Human Condition	Lab Conditions	SGV [1]/TCV [2] PFU [3]	C [4]	G [5]	P [6]	Effect	Reference
Reactivation after immunosuppression	i.p. inoculation of C3H/St mice.	SGV subcutaneous infection with 10^3 PFU Latency = 8 months	x			Immunosuppression by rabbit antilymphocyte serum and corticosteroid.	[65]
Pneumonitis	Intranasal MCMV plus cyclophosphamide 24 h after viral inoculation in BALB/c.	SGV, 10^5 PFU	x			Interstitial pneumonitis.	[66] Reviewed in [67]
Atherogenesis	MCMV inoculated i.p. in APO-E−/− mice (C57BL/6 background).	SGV 10^5 PFU Or 3 × 10^4 PFU TCV		x		Larger atherosclerotic lesions in infected mice, potentially caused by upregulation of p38.	[68][69]

Human Condition	Lab Conditions	SGV [1]/TCV [2] PFU [3]	C [4]	G [5]	P [6]	Effect	Reference
Transplant-arteriosclerosis	Human peripheral blood leukocyte/Rag-2−/−γc−/− mouse-xenograft-model inoculated with HCMV.	Segments of mammary artery incubated in vivo with 10^5 PFU HCMV before implantation		x	x	Transplant arteriosclerosis was significantly elevated and increased ICAM-1, PDGF-R-b and macrophages.	[70]
Reactivation of CMV infection post organ transplantation	Immunosuppression of BALB/c mice receiving donor kidney (C57BL/6 allograft) leads to dissemination of reactivated MCMV.	SGV 10^7 PFU MCMV-Δm157. Mice used as donors 4–8 months post i.p. inoculation (latency)	x		x	Two-step process: allograft ischemia and reperfusion injury (step 1). Immunosuppression mediated viral dissemination (step 2).	[71]
Sjogren's syndrome	1. MCMV inoculated i.p. in *Fas*-deficient C57Bl/6-lpr/lpr mice. 2. MCMV inoculated i.p. in tumor necrosis factor-related apoptosis-inducing ligand deficient BALB/c mice.	SGV, 10^5 PFU SGV 10^4 PFU		x x		Salivary gland inflammation and autoantibody production. Autoantibody production and lymphocytic aggregates.	[72]
Reactivation of CMV infection post haemopoietic cell transplantation	1. BALB/c mice undergo sublethal irradiation (6 Gy), undergo syngeneic haematopoietic stem cell transplant 6 h later, 2 h later inoculated with CMV via foot pad.	TCV 10^5 PFU			x	Pulmonary infection control depends on CD8 T cell reconstitution.	[73]
	2. CD8 T cell immunotherapy.					Controls CMV associated immunopathology.	[74] Reviewed in [59]
Graft versus host disease	1. Inoculated with MCMV 3 days prior to transplant. A variety of H-2 defined mice inoculated i.v. with spleen cells (either MHC1 or MHCII disparity).	1 × LD_{50} SGV		x		Reduction of CTL and immunodeficiency induced, 10 × less donor cells required.	[75]
	2. Strain specific antibody therapy. Latently inoculated mice (i.p).	10^4 (B6 or B6D2F1) or 5 × 10^3 PFU (BALB/c) SGV		x		Strain-specific antibody therapy protects from MCMV reactivation.	[76]
CMV retinitis	1. Inoculation into supraciliary space (described in[77]) + immunosuppression of BALB/c mice via methylprednisolone every 3 days starting 2 days prior to inoculation.	SGV 5 × 10^3 PFU	x			Retinitis abrogated using i.v. siRNAs directed against MCMV immediate early protein-3 (IE-3).	[78][79]
	2. Immunosuppression using C57Bl/6J intraretinal inoculation PLUS MAIDs.	SGV 10^4 PFU	x			(MAIDS—retrovirus mixture defined in [80]) Severity of effect may be due to suppressor of cytokine signaling (SOCS) 1 and 2.	[81]

Human Condition	Lab Conditions	SGV [1]/TCV [2] PFU [3]	C [4]	G [5]	P [6]	Effect	Reference
Renal allograft loss due to MCMV reactivation (donor positive)	Donor BALB/c recipient C57Bl/6 after renal transplantation.	TCV MCMV or Δm157, Infection with 10^4 PFU (Δm157) all donors. Recipient either 10^4 PFU Δm157/WT or 10^2 Δm157.		x	x	Th17 inhibition reduced injury to graft.	[82]
Brain infection in immunosuppressed patients	Severe combined immunodeficient (SCID) mice (BALB/c background), intracranial infection. Virus expressing green fluorescent protein (GFP).	TCV, 10^6 PFU (GFP), 4.83 × 10^5 PFU wildtype		x	x	Adoptive transfer of MCMV-specific CD4 T cells clears CMV from the brain. Treatment prior to infection prevents MCMV replication.	[83]
Hemophagocytic lymphohistiocytosis	IFN-γ-knockout (KO) mice on BALB/c background or BALB/c	SGV, 5 × 10^3 PFU		x		Severity not associated with titer, associated with inflammation.	[84]
Laboratory diagnosis of hepatitis	i.v. inoculation of C57BL/6 mice with marker virus (luciferase, mCherry, SINFEKL)	TCV, 10^6 PFU		x		Blood biochemistry levels given (allowing diagnosis of hepatitis).	[85]

[1] Salivary gland-derived virus. [2] Tissue culture-derived virus. [3] Plaque-forming units. [4] Chemical modification e.g., chemotherapeutic. [5] Genetic modification of mouse or virus. [6] Physiological treatment (e.g., surgery).

5. MCMV Exacerbates the Effects of Other Clinical Diseases

For some models, the addition of MCMV can exacerbate disease, reflecting human disease particularly in the intensive care unit setting. These are generally diseases with an immune modulation component. These models are listed in Table 3.

Table 3. Models demonstrating MCMV-associated effects on other diseases.

Medical Condition	Lab Conditions	SGV [1]/TCV [2] PFU [3]	Effect	Reference
Ulcerative colitis in CMV-inoculated individuals	T cell receptor alpha−/− mice (C57BL/6 background) inoculated i.p. at 7 days of age with MCMV or MCMV-enhanced GFP	TCV, 5 × 10^5 PFU of MCMV for C57BL/6 mice and 2 × 10^4 PFU of MCMV-EGFP for TCR-alpha−/−	Ulcerative colitis is exacerbated in latently infected mice	[86]
Growth of glioblastoma	Mice inoculated i.p. day 2 of life (P2), tumor injection week 15	TCV 10^3 PFU MCMV-Δm157	Tumor growth and reduction in survival	[87]

Medical Condition	Lab Conditions	SGV [1]/TCV [2] PFU [3]	Effect	Reference
CMV reactivation after physical damage	Caecal ligation and puncture in latently i.p. inoculated BALB/c mice	TCV, 2 × 10^4 cgrmPFU	Plus *S. aureus* to induce bacterial pneumonia	[88][89]
MCMV infection after cholestasis	C57BL/8 mice bile duct ligated, inoculated i.p. Δm157-MCMV-luciferase	SGV/TCV not specified. 2 × 10^5 PFU	Impaired inflammatory response, but no increase in liver pathology	[90]
Melanomas growth, repeated injection recapitulates transient response	Intra-tumoral inoculation of MCMV or ΔgL MCMV (spread deficient) impairs melanoma growth in BALB/c mice	TCV (described in [91]), 5 × 10^5 PFU	Infection of macrophages leads to proinflammatory M1 state	[92]
Idiopathic pulmonary fibrosis	Latent MCMV infection in BALB/c mice (i.p.—4 weeks prior), intratracheal bleomycin	SGV, 10^5 PFU	MCMV-exacerbated fibrosis, activation of TGF-β1	[93]
Experimental autoimmune encephalomyelitis (EAE)	C57BL/6J and CD80/86−/− mice inoculated with MCMV i.p. and 8 days later, EAE induced	SGV, 5 × 10^4 PFU	More severe disease (e.g., enhanced demyelination), severity associated with number of splenic $CD4^+CD28^{null}$ T cells	[94]
Use of bronchiolar lavage (BAL) to detect reactivation of CMV in sepsis	BALB/c mice inoculated with MCMV, sepsis by caecal ligation and puncture	SGV/TCV not specified; 10^2, 10^6	qPCR of BAL cell pellets similar to that of lung tissue	[95]
Acute colitis	C57BL/6 mice	TCV, 3 × 10^4 PFU	Acceleration of colitis development, but no difference in histology	[96]
Allergic airway disease	C57BL/6 mice inoculated intra tracheally	TCV, 10^6 PFU	Exposed to ovalbumin, CMV-exacerbated disease	[97]

[1] Salivary gland-derived virus. [2] Tissue culture-derived virus. [3] Plaque-forming units.

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